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HYDROGEN-ION CONCENTRATION OF PLANT JUICES

I. The Accurate Determination of the Hydrogen-ion Concentration of Plant Juices by Means of the Hydrogen Electrode[1]

CLINTON B. CLEVENGER

From the Departments of Soils and Agricultural Bacteriology, University of Wisconsin, Madison, Wisconsin

Received for publication August 25, 1919

INTRODUCTION

The hydrogen electrode has but recently been employed for the measurements of the hydrogen-ion concentration in plant juices. This method has been used for this purpose by Haas (7), Kappen (10), Hempel (9), and Truog and Meacham (22). The type of apparatus used by these workers gives values of sufficient accuracy for work in which small differences are not significant. The simple type of gas chain is also to be preferred in the electrometric titration method for total acidity in plant juices as used by Haas (8).

The hydrogen electrode was studied critically by Loomis and Acree (11) and applied by them (12) to accurate measurements in organic reactions. Clark (2) has adapted the method for accurate measurements in the case of bacteriological media. Because of the great importance of acidity[2] in plant juices it is desirable to adapt the method to the accurate measurement of this acidity. That the acidity may exert marked influences upon the activities of the plant is evident from the work of Reed (17, 19) and Bunzell (1). These investigators found that a slight acidity inhibits the action of oxidases which are now held to be universally distributed (18) in plants and play an essential rôle in plant respiration. According to Bunzell (1) the concentrations of acidity which completely inhibit the oxidase action for each type of plant material fall within a rather narrow range.

That enzymes are affected markedly by acidity is noted further in the observation of Michaelis (16) that the natural acidity of body fluids containing an enzyme is, as a rule, the optimum acidity for the activity of that

[1] Part I of thesis submitted to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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This opportunity is taken to express to Professor Truog and Professor Fred, under whose direction this work has been done, due appreciation for their suggestions and criticisms.

[2] Acidity and hydrogen-ion concentration are used synonymously in this paper.

enzyme. Sherman, Thomas and Baldwin (21) have found that amylases representative of the starch-splitting enzymes of the higher animals, higher plants and of fungi, respectively, have an optimum acidity for their greatest activity. Kappen (10) believes that in plants, as in animals, slight changes in acidity influence the activity of the enzymes which are present. In this way the important life processes of the plant are affected. Crocker (4) has suggested that changes in acidity may be important in the regulation of

TABLE 1

Representative measurements showing possible accuracy of the apparatus and method when buffer solutions and plant juices are used

SOLUTION	ELECTRODE VESSEL	TIME			POTENTIOMETER READINGS IN VOLTS		TEMPERATURE	P_H VALUE
		H_2 started	Shaking began	Readings taken	Readings	Average of first readings		
Buffer solution of KH_2PO_4 + NaOH	A	11:45	11:57	12:01	0.68739	0.68738	31.5°	5.801
				12:05	0.68743			
	B	11:45	11:57	12:02	0.68738			
				12:06	0.68736			
Buffer solution of KH_2PO_4 + NaOH	A	12:35	12:51	12:55	0.68834	0.68837	32.3°	5.809
				12:58	0.68844			
	B	12:35	12:51	12:56	0.68841			
				1:01	0.68852			
Juice of medium red clover	A	3:47	4:00	4:10	0.70977	0.70973	25.0°	6.276
				4:13	0.70973			
	B	3:47	4:00	4:12	0.70969			
				4:15	0.70972			
Juice of soybean seedlings	A	2:15	2:27	2:31	0.69931	0.69928	31.7°	5.965
				2:35	0.69928			
	B	2:15	2:27	2:33	0.69926			
				2:38	0.69925			

transpiration. This suggestion is made from the work of MacDougal (14) and MacDougal and Spoehr (15) on the effects of acids and bases on imbibition of water by plant tissues and plant gels. It seems possible that certain conditions may change the acidity of the plant juice sufficiently to produce an acidity which is unfavorable for the plant.

In a study of the factors affecting the acidity of plant juices the possible effect of soil acidity is one of considerable importance. Measurements made by Truog and Meacham (22) bear upon this relation These results indicate

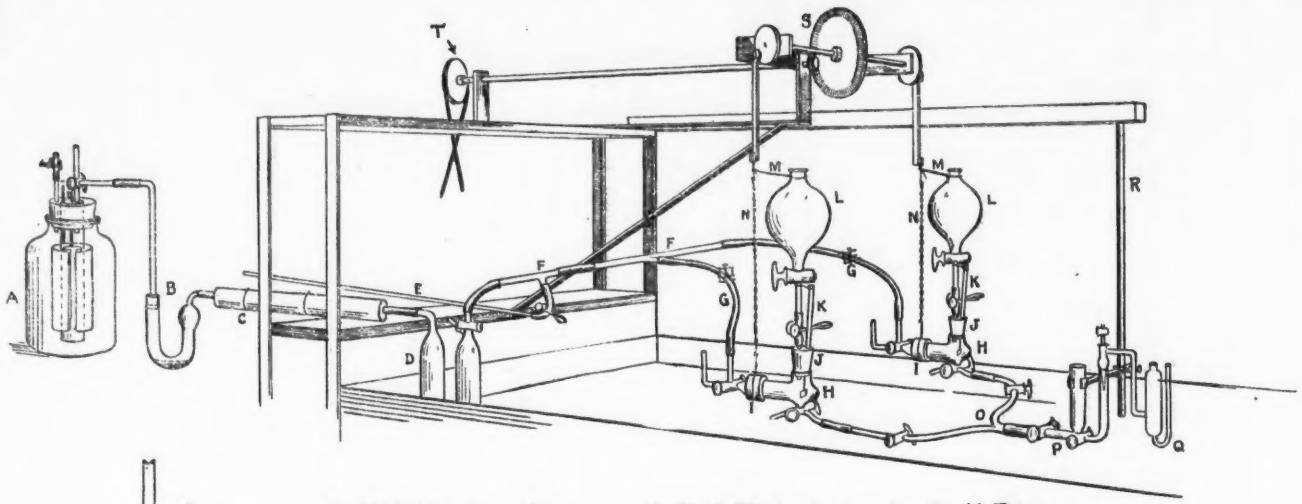
that the acidity of the plant juice may often be increased by an acid reaction of the soil. Since differences caused by such factors may in many cases be quite small and yet be of great importance, it seemed desirable to adapt the hydrogen electrode method of measurement to the determination of plant acidity so as to get much more accurate values of plant acidity than have previously been obtained. A procedure and apparatus which will give accurate values of acidity with one kind of material may not always work similarly with other material. The Clark and Lubs (3) procedure and apparatus gives accurate values with bacteriological media but do not always give accurate values with plant juices. It is probable that substances present at least in some plant juices are the cause of considerable difficulty (6).

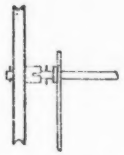
THE APPARATUS

The apparatus used in the present investigations is similar in its essentials to the chain described by Clark and Lubs (2) which embodies the use of the Clark electrode vessel, devised for shaking, a constant-temperature air-bath, electrolytically prepared hydrogen and sensitive measuring instruments. In adapting this form to the present work certain important modifications in the electrode vessel, in the shaking apparatus and in the bath were found advantageous. The electrode vessel was modified to overcome the difficulties produced by foaming of the juices. A cheaper and more convenient shaking apparatus was devised. A less expensive bath which is also more convenient was used. Although the electrode vessel is not maintained at a constant temperature, the slow fluctuation of the temperature of the air makes possible a temperature correction without appreciable error.

Electrode vessel

A 30-cc. dropping funnel *L* is attached to the hydrogen electrode vessel *H* as shown in the figure. The dropping funnel is attached with rubber tubing to a small glass tube which passes through the stopper *J* that fits into the electrode vessel. The funnel is further supported by means of a wire loop *M* fastened to and extending from the support *N* holding the electrode vessel. When the funnel is removed a pinch-cock is placed on the rubber connection to close the outlet. Plant juices, excepting those of relatively high acidity, foam considerably when hydrogen is rapidly passed through them, which results in the expulsion of considerable liquid from a small cell like the Clark vessel. By placing the liquid in the dropping funnel which is considerably wider than the cell, loss during saturation with hydrogen is prevented. An additional advantage of this dropping funnel is that it greatly lessens the period of contact between the liquid and electrode, and probably facilitates saturation of the electrode by preventing the formation of a film over its surface.





SIDE VIEW OF T

A - Hydrogen Generator
B - Tube containing H_2SO_4
C - Electrical heating unit
D - U-Tube for cooling hydrogen
E - Hydrogen overflow
F - T-Tubes
G - Hydrogen Leads
H - Clark Electrode vessels
I - Rubber Rings
J Rubber Stoppers
K - Hydrogen Electrodes
L - Dropping Funnels
M - Funnel Supports
N - Electrode vessel supports
O - Y-Tube
P - Intermediate vessel of KCl
Q Calomel Electrode
R Support for P
S Bevel Gear of Shaking Device
T - Metal Pulley

FIG. 1. THE MAIN PARTS AND THEIR ARRANGEMENT OF THE HYDROGEN ELECTRODE APPARATUS

SHAKING APPARATUS

At the place of suspension each electrode vessel is provided with a rubber ring *I* for insulation. Each electrode vessel is suspended by a chain *N* to the metal wheels mounted on the ends of a shaft 4½ inches long. This shaft is driven by a bevel gear as shown in the figure at *S*. In order that the shaking apparatus may be started and stopped without stopping the motor which also runs the water stirrer in the bath, the metal pulley *T* is arranged to slide in and out of gear as shown in the figure. The cross pin in the shaft fits into notches on the inside collar of the pulley. By pushing the pulley inward the shaking apparatus is set in gear and by pushing it outward the apparatus is put out of gear. The rate of shaking of the electrode vessel should be about one cycle per second.

Connecting vessel

The duplicate electrode vessels are joined by a glass Y-tube *O* to the same intermediate vessel *P* of saturated KCl solution. This vessel is held in position by the rigid support *R*. A cord supporting the Y and attached to an arm from *R* is not shown in the figure. The Y-tube is provided with a cock in each of the two arms.

Constant temperature bath

A constant-temperature air-bath is not used. The hydrogen electrode element and connecting vessel of the chain are suspended above a constant-temperature water-bath maintained at 25°C. In this bath the calomel electrode battery and comparison cell are kept. Since water is a fairly good conductor and stray currents from the wires, motor and other sources may play upon the bath, it is not desirable to operate the hydrogen vessel in the water. Electrical connection is often made between the chain and the water, and disturbances might easily be set up. At each measurement the temperature of the air above the bath is taken and correction made.

Calomel electrode battery

The calomel electrode battery[3] which is not shown in the figure is made up of five cells with a comparison cell *Q* joined by a ground-glass connection. These cells are closed with cork stoppers which are covered with a mixture of paraffin and beeswax to make tight seals. In the preparation of the chemicals the procedure given by Loomis and Acree (11) was followed. Mercury redistilled twice and N/10 KCl solution were used.

The comparison calomel electrode *Q* fits into the intermediate vessel by a ground-glass joint. During a measurement it is kept at constant temperature by placing it in a jar of water which sets in the bath and is raised sufficiently for this purpose.

[3] Devised by Dr. N. E. Loomis for the Eli Lilley Company.

Hydrogen generator

The hydrogen is generated electrolytically in *A* from a 10 per cent solution of KOH, nickel electrodes being used. Ten to twelve amperes of current are employed. This amount generates sufficient hydrogen for saturation of duplicate samples at the same time. The hydrogen first passes over H_2SO_4 in the glass tube *B*, then through heated palladiumized asbestos in *C* and finally through a glass U-tube *D*. The latter extends into the constant-temperature bath by which means the hydrogen is cooled. By means of two small T-tubes following the U-tube, a lead *G* to each hydrogen electrode vessel and an outlet *E* for waste hydrogen are provided. The latter outlet consists of a glass tube of smaller bore than the T and is closed by a pinch-cock at the rubber connection near the T. A glass cock in the connection from the U-tube closes the system when not in use. It was not found necessary to keep a small current running overnight to prevent diffusion of air into the generator. Although some hydrogen diffuses out from the system, any effects due to entrance of air when the sytem is closed, have not been noticed. All rubber connections are made tight by a coating of paraffin and beeswax.

Preparation of palladium electrodes

Platinum electrodes coated with palladium black are used. The black is deposited by electrolysis from a 2 per cent solution of $PdCl_2$ containing a trace of lead acetate. When sufficient black has been deposited the electrodes are placed with the current running, first into a dilute solution of H_2SO_4. This electrolyzes $PdCl_2$ adhering to the black and removes any occluded chlorine by displacement with hydrogen. They are then dipped into a dish of distilled water. The whole process of preparation requires about $1\frac{1}{2}$ minutes. Finally, the blackened electrode is thoroughly rinsed with running distilled water and is then ready for use.

In order to keep the $PdCl_2$ solution, which is used for depositing the black, free from contamination of substances from the plant, the black from the electrodes is not returned to the solution. It appears that in work with inorganic solutions special precautions (11) with regard to keeping the electrolyzing solution free from contamination are not necessary. With material like plant juices it seems that contamination from them might make it difficult to secure good electrodes. In cleaning, the electrodes are first rinsed to remove adhering plant juice and then let stand in a beaker of distilled water. This dissolves out further amounts of juice. The black is wiped off with a clean piece of ashless filter paper and the electrode is ready for recoating. At frequent intervals the electrodes are cleaned by placing in concentrated HNO_3. This dissolves the palladium, leaving a clean platinum surface.

Measuring instruments

The measuring instruments consist of a Leeds and Northrup improved type of potentiometer, a sensitive D'Arsoval galvanometer and two Leeds and Northrup Weston cells. One of the latter was compared with a cell standardized by the United States Bureau of Standards. The value of the comparison calomel electrode was checked frequently with electrodes of the battery.

PROCEDURE OF MEASUREMENT

Preparation and flooding of electrodes

Freshly prepared electrodes are always used in making a determination. These are placed into the electrode vessels and the dropping funnels with pinch-cocks are then attached. The cocks in the funnels are closed, and the vessels, tilted so the inlets are higher than the outlets, are flooded with hydrogen. The electrode vessels are flooded separately by running the hydrogen into each for about five minutes.

Extraction of plant juices

During this interval the plant material is usually cut and the sample of juice extracted. The plant tissue is first macerated by passing through a tinned meat chopper. The macerated tissue is placed on a clean muslin cloth which is spread on the bottom plate of the press which is used. The edges of the cloth are folded over each other enclosing entirely the tissue. The juice is then pressed out by means of pressure. The pressure should be uniform for different samples. The cloth should be a good grade of bleached muslin thoroughly washed and cut into squares of convenient size. A square is used but once. The cloth retains coarse colloidal and other matter, giving a juice from which on centrifuging for 10 minutes no material except salts are thrown out. The centrifuging is thus omitted.

Saturation of juice with hydrogen

Three to five cubic centimeters of the juice are placed in each of the dropping funnels. The waste outlet of the electrode vessel is closed and the cocks in the funnels opened slightly. The hydrogen is made to bubble evenly through the juice by regulation of screw clamps on the rubber leads to the vessels. The hydrogen should bubble through for about 10 minutes with the amounts of juice indicated.

No difficulty is experienced from foaming with this arrangement except when the juices approach the neutral or alkaline point. In such cases a long peaked cone of filter paper inserted snugly into the opening of the funnel is of material help. This serves to break the bubbles, permitting the hydrogen to escape. Ashless filter paper is used and pieces made by cutting the 9-cm. size in halves are convenient.

Transfer of juice to the electrode vessels

After saturation the cocks in the funnels are closed and the current producing the hydrogen is reduced from 10 amperes to about 1 ampere. This reduces the large hydrogen stream which is now not needed and the hydrogen generator is not unnecessarily heated. The exit for overflow of hydrogen is opened by releasing the pinch-cock. The cocks in the funnels are opened and the juice allowed to flow into the electrode vessels. The cocks are closed when a little liquid still remains in the stems of the funnels which prevents the possibility of the entrance of air into the vessels.

Shaking electrode vessels

The electrode vessels are gently shaken for 2 minutes. Long violent shaking sometimes appears to discharge the electrodes, while with no shaking a local equilibrium may be obtained, as is indicated by a higher E. M. F. of the chain. The period of 2 minutes gave good results and was arbitrarily chosen.

Making the reading

In making the measurements only the N/10 calomel electrode is used as shown by the following combination:

PdH_2 Plant Juice//Saturated KCl//N/10 KCl HgCl Hg

The electrodes are left at least half exposed to the hydrogen gas. Fresh saturated KCl solution is run into the connecting tube of the electrode vessel as in the usual procedure (2). A rubber tube carries away the overflow of KCl solution from the outlet. The calomel electrode and the wires leading to the potentiometer are put into place. Contact between the juice and the saturated KCl is made by the film of KCl solution around the stopcock, which is left ungreased around its middle portion. A good contact is insured if a scratch connecting with the bore is made around the cock. Readings are then taken immediately. Constant potentials are maintained most generally for 2 to 5 minutes and often much longer. Duplicate measurements usually agree within 0.1 millivolt.

Although more contact potential is ordinarily set up with small contact surfaces (5) yet this may be the opposite when working with plant juices. In several trials when the contact was brought below the bore of the cock into the large tube a contact potential developed quite rapidly. This rapid development of contact potential with freshly extracted plant juices may be due to the reaction of the KCl with the plant juice. The N/10 KCl of the calomel cell which comes in contact with the saturated solution is renewed after each measurement.

The acidity of plant juices may change appreciably on standing, as is indicated in the second part of this paper; hence it is necessary to make the measurements within as short a time as possible. Since two sets of comparative determinations can be made in an hour, corrections for changes in vapor and barometric pressure (13) have not been taken into account.

Accuracy of the method

One of the tests which gives value to a method lies in the reproducibility of results. This is particularly true where comparative results are wanted. Not only should duplicate measurements, which are made at the same time and thus under the same conditions agree, but measurements made at different times also should agree. In the case of the buffer solution of KH_2PO_4 + NaOH, measurements at different temperatures, as given in table 1, show good agreement. Examples of measurements and constancy of potentials, which are respresentative of the measurements of plant juices of the following paper, are given. The good duplication of readings between the two hydrogen electrodes in separate cells with the same juice is to be especially noted.

SUMMARY

The importance of the acidity or hydrogen-ion concentration of plant juices, and the need of developing an accurate method for its determination have been pointed out.

An accurate method for this purpose has been developed in which are used: The Clark electrode vessel, modified and adapted for work with plant juices, a simple means for shaking the vessel, a constant-temperature water-bath in which the calomel electrodes are kept, electrolytically prepared hydrogen, and accurate measuring instruments. Corrections for temperature of the air are made when readings are made. Contact of the electrodes and plant juice during saturation with hydrogen is prevented by placing the latter into dropping funnels attached to the electrode vessels. By this means contamination of the electrodes by the juice during saturation with hydrogen is prevented. The electrodes are always recoated for each determination.

The plant juice is prepared by macerating the tissue and expressing the juice by means of a press. The macerated tissue is wrapped in a clean muslin cloth, which gives a juice free from coarse colloidal or other material.

Contact is made between the plant juice and saturated KCl solution by means of a scratch around the cock connecting the two. This reduces the contact potential which develops quite rapidly when the connection is made by opening the cock wide.

Duplicate measurements usually agree within 0.1 millivolt and constant potentials are maintained for several minutes or longer. A table showing the possible accuracy is given. These results are undoubtedly more accurate than any previously reported on plants.

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HYDROGEN-ION CONCENTRATION OF PLANT JUICES

II. Factors Affecting the Acidity or Hydrogen-ion Concentration of Plant Juices[1]

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INTRODUCTION

The importance of the hydrogen-ion concentration of plant juices was noted in the first part of this paper. Since many of the important reactions and processes occurring within the plant are influenced by slight changes in acidity, it is desirable to know something of the factors which produce such changes. In plant juices as in any medium the hydrogen-ion concentration depends upon the kind and amounts of acids, the nature of the solvent or medium and the buffer substances present. The hydrogen-ion concentration which an acid can produce depends upon its degree of dissociation and upon the amount of acid that is present. Organic acids are, as a rule, much less dissociated than inorganic acids and hence with equal dilutions of equivalent amounts of acids less acidity is developed by the former than by the latter.

Explanation of buffer action

Buffer substances act as regulators of the reaction preventing rapid fluctuations due to an increase or decrease in total acids. Buffer action may be illustrated in the following way. In a solution of HCl there is, according to the mass-action law, an equilibrium between the ions and the undissociated molecules which may be represented by the equation:

$$\frac{C_{H+} \times C_{Cl-}}{C_{HCl}} = K$$

This equation states that the product of the concentrations of the hydrogen and chlorine ions divided by the concentration of the undissociated HCl is

[1] Part II of a thesis submitted to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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This opportunity is taken to express to Professor Truog and Professor Fred, under whose direction this work has been done, due appreciation for their suggestions and criticisms.

equal to a constant K. If to the solution more HCl is supplied the concentration of HCl molecules is increased and since the value of K is constant it is necessary, if the equation is to hold true, that the product of the concentrations of the hydrogen and chlorine ions also be increased. In order that the product may increase, more of the HCl dissociates to give greater concentrations of both the hydrogen and chlorine ions. If however, a salt of a weak acid as CH_3COONa is added to the solution the concentration of the hydrogen-ions is held down to some extent when more acid is added. Sodium chloride is formed and the acetate-ions which are supplied unite with hydrogen-ions present to form CH_3COOH molecules which dissociate much less than HCl molecules. Because of this slight dissociation of CH_3COOH the concentration of hydrogen-ions is lowered in an extent which depends upon the amount of CH_3COONa which is present. The CH_3COONa serves as a buffer in this case.

In plants organic salts, proteins and other substances may serve as buffers in the way just explained. Hempel (10) has investigated the buffer processes in succulent plants. She concludes that on the acid side of the litmus end-point, normal malate is the main buffer and on the alkaline side of the litmus end-point aluminum malate and some other unknown substances are the principal buffers. These buffer substances play a very important rôle in plant life because they prevent rapid and excessive changes in the acidity of plants which would otherwise result from factors mentioned in the following discussion.

Acids found in plants

According to Pfeffer (20) every plant produces some organic acid either free or in the form of an acid salt. Oxalic, malic, citric, and tartaric acids occur most frequently. With the exception of the oxalic acid present as calcium oxalate, the organic acids and their salts are for the most part held in solution. The inorganic acids present in the plant are taken up from the soil. The distribution of the acids in the plant (1) has been found to vary with the different parts as stems, leaves, and flowers.

Formation of organic acids in plants

The formation of organic acids in plants is now commonly held to be connected with the cell respiration processes. Pfeffer (20) states that the acids are usually products of katabolism, but probably may also be produced synthetically. Kraus (13) believes that these acids are secondary products from the splitting up of proteins. Astruc (2) states that production of acids is intimately associated with processes of respiration and assimilation. The acidity in succulents he says is due chiefly to malic acid. Later workers, as Long (15), MacDougal, Long and Brown (17), hold the view that acids are formed from the metabolism of carbohydrates. Factors, as water supply,

light, and temperature, which influence the formation of carbohydrates and sugars will indirectly influence the production of these acids. In succulent plants MacDougal, Richards and Spoehr (18) attribute acid formation to imperfect oxidations resulting from the lessened aeration in the interior of massive tissues. Considerable work has been done by Richards (22, 23, 24, 25, 26) on acidity and the gas interchange of oxygen and carbon dioxide in cacti. Richards (25) states that the partial oxidation processes which give rise to acids of which undoubtedly the chief one is malic can be conceived as taking place at all times. The substance oxidized is believed to be the complex of the living protoplasm itself, which in turn draws upon the reserve food supply of accumulated carbohydrates. It is likely that sugars form unstable compounds with the living protoplasm and in such unions oxidation and release of energy take place. Whether free oxygen is necessary for this process Richards does not know, but quotes Astruc (25) as stating that acids are best produced in an atmosphere rich in oxygen. Purjewicz (21) has pointed out that in the breaking down of acids which he regarded as an oxidation process, in the absence of oxygen this process is greatly inhibited. It appears thus that free oxygen is needed for both processes. However, Richards (25) is inclined to doubt that free oxygen is needed for succulents in which cases auto-oxidations by intramolecular respiration might occur.

An early view of acid formation, but which found little favor among investigators, was that of Liebig (14). Liebig thought from the disappearance of organic acids in the ripening of fruits, that these acids were concerned in the building up of carbohydrates and constituted intermediate products between the carbon dioxide on the one hand and sugar on the other. In view of the fact that acids are formed and accumulate within the plant during the night when photosynthesis of carbohydrates does not occur, this theory at once becomes untenable.

Concerning the period of growth of the plant in which most acidity is produced, Aubert (3) found less acid in the young parts and most in the recently mature tissue. According to Astruc (2) the acids are formed most in young organs which show high cellular activity and a maximum of turgescence. The acids gradually diminish in amount as the tissues advance in age either by uniting with bases absorbed from the soil or by esterification. Nicolas (19) states that "in young organs, principally leaves, intramolecular combustions are more complete than in older organs; young tissues consume much more oxygen than those completely developed, ... and thus liberate greater quantities of energy which they use in growth." This statement is in harmony with the observations of Aubert just noted as to formation of acids if they are produced from incomplete oxidation processes.

Acid formation in plants appears to be intimately connected with the living protoplasm. Although the acids may be largely derived from transformations of carbohydrates, it seems that for breaking up of carbohydrate molecules with liberation of energy the molecules must first form some sort of union

with the living protoplasm. How far the processes for securing energy from the carbohydrates are removed from those in which the proteins are formed and broken down is not known. In all these processes the protoplasm is concerned. Whether acid formation is regarded as a result of incomplete oxidation of carbohydrates or from the formation and decomposition of proteins or all of them depends largely upon the way in which the metabolic processes of the plant are viewed.

Factors influencing acid accumulation

The factors which influence the accumulation of acids may be divided into two groups: viz., internal and external factors. Considering the internal factors, the accumulation of acids depends upon the kind and age of the plant and the removal of the acids by oxidation or precipitation as salts. The chemical reactions of the plant are governed by the same factors affecting chemical reactions in the laboratory, such as temperature, concentration, and nature of the reacting substances. The formation of calcium oxalate is an example of the removal of an acid as an insoluble salt. Salts of other acids are usually more soluble and their production occurs to some extent in a self-regulatory manner, since in the higher plants they are commonly used in the maintenance and regulation of turgor (20). Pfeffer (20) states that a diminution in acidity of the cell sap may be produced by an increased formation of alkali, by a consumption of acid, or by union with basic substances.

The external factors which influence most the accumulation of organic acids are light and temperature. That high illumination and high temperature produce rapid destruction or decomposition of these acids has been found by many investigators. Heyne (11) was the first to note that leaves had a more acid taste in the morning than late in the afternoon. Kraus (13) attributed the loss of acid during the day to the direct effect of light. De Vries, who worked upon the periodicity of acid formation in succulents, is reported (25) to have found that prolonged darkness as well as exposure to high temperatures produces a loss of acids. De Vries regards the formation and splitting up of the acids as coincident processes which are always taking place, the former being more rapid than the latter at night. Warburg (31) believed that the decomposition of the acids during the day furnished a source of carbon dioxide for photosynthesis in plants which were not favorably situated as to gas-interchange relations. Aubert (3), Purjewicz (21) and Astruc (2) also found this periodicity of formation and destruction of acids to be due to light and temperature changes. Pfeffer (20) states that the percentage of acids decreases to about the same extent when the temperature of a plant in darkness is raised from 15° to 45°C. as when it is exposed to daylight.

Probably the work of Spoehr (27, 28, 29) on the photolysis of plant acids has been the most extensive as regards their chemical decomposition. Spoehr (28) working in vitro with chemical preparations found that acetic, glycolic, propionic, malic, tartaric and citric acids are decomposed in light, giving off

considerable quantities of carbon dioxide. In general the acids in the form of salts are broken up more easily. The decomposition of malic acid and its products was studied (27) in particular. This acid gave the following products: formaldehyde, acetaldehyde, formic acid, acetic acid, glycolic acid, oxalic acid, and carbon dioxide. It is thought that in the plant these products can also be formed by the influence of light. From the malic acid, carbon dioxide is split off, giving rise to alcohols which in presence of air are oxidized to aldehydes and these, in turn, to acids. In this way the formation of oxalic acid may be explained. Although the latter acid is easily decomposed by light, its calcium salt is photochemically a stable compound. It is also pointed out that increases in volatile acids, as acetic and formic, can be explained in this way.

With plant juices Spoehr found that deacidification is not due to action of enzymes, neither is it wholly dependent upon the living protoplasm, since expressed juice when placed in sunlight diminishes in acidity with formation of carbon dioxide. Whether the latter conclusion is correct may be questioned since fresh plant juice undoubtedly contains living protoplasm.

Long (15), in working with large cacti, found a higher accumulation of acids in the outer than in the inner regions. This higher amount of acids, he says, is probably due to the higher concentration of sugar in the outer parts. In these parts sugar metabolism is greatest and hence a greater production of acids results. Since the outer parts are most exposed to the heat and light, it is here also that the greatest fluctuations due to diurnal changes occur. In what manner the acids are produced in the metabolism of sugars is not made clear.

The effect of the kind of soil and its fertilizer treatment on plant acidity is beginning to receive considerable attention. The effect and relation of the soil medium upon the plant (12) and acidity within the plant appears to be of considerable importance. Reference to this relation has already been made in Part I of this paper (page 217) in referring to the investigations of Truog and Meacham. Charabot and Hebert (5) found that with the peppermint plant, mineral salts when added to the soil generally increased the amount of volatile acid in the fresh leaves.

The effect of soil treatment is further shown in the work of Bauer and Haas (4). Their results indicate that there is a direct correlation between the acidity of the soil and that of the corn plant.

The observation of Comes (7) of the relation existing between the acidity of the cell sap of wheat and its resistance to rust is of interest. Comes found that with an increase in starch in the grain there is less acidity in the cell sap, making the plant more susceptible to rust attacks. Since with the increase in amounts of starch the percentage content of protein is lowered, this observation strengthens the view that acids are formed as by-products in protein metabolism. It is thus evident that factors like soil treatment, which influence the production of starch, may have an effect upon the acidity.

EXPERIMENTAL

The measurements reported herein were all made by the method and apparatus described in Part I of this paper. The factor studied first was the effect of time of standing of expressed juice and of plants which had been cut. In work of this nature it is important to know something of the rapidity of the changes in acidity due to cutting of the plants and standing of the extracted juice in order to eliminate errors from these sources.

Effect of standing on acidity of juice and plants

The results of the effect of standing on the acidity of extracted juice are given in table 1. Alfalfa juice was used. This table shows that the extracted juice suffers a change on standing which may be in either direction. In table 1

TABLE 1

Effect of standing on the acidity of plant juice expressed in P_H *values*

(a) Plants cut 2:35 p.m.

Time of standing, minutes	0	80
P_H value	5.935	5.930

(b) Plants cut 2:00 p.m.

Time of standing, minutes	0	45	135
P_H value	5.790	5.816	5.758

(c) Plants cut at 7:15 a.m.

Time of standing, minutes	0	50	115
P_H value	5.900	5.886	5.753

(a) a slight increase in acidity has occurred. In table 1 (b) the juice is shown to become less acid during the first hour of standing. This is followed by an increase in acidity 90 minutes later, which acidity is greater than that of the fresh juice. In table 1 (c) the acidity increases directly on standing.

This increase in acidity is probably due to enzyme action on the sugars and other changes in the protoplasmic substances present. The decrease in acidity of the juice in table 1 (b) is probably due to the breaking up of acids present as noted by Richards (25). In this case it appears that at first the rate of acid destruction was greater than the rate of acid formation.

The changes in acidity of the juice of plants which have been cut and let stand in the laboratory were uniformly toward less acidity, with one exception. Table 2 gives these results. It is to be noted that these changes correspond to the diurnal changes in acidity of plants during the day due to such factors as light and temperature.

The results in tables 1 and 2 show that acidity changes do occur in extracted juices and plants that have been cut. Although these changes may be small, yet it is clearly shown that determinations of acidity should be made as quickly as possible after the plants are cut and the juice is extracted.

TABLE 2

The effect on acidity of allowing cut alfalfa plants to stand before expressing the juice

(a) Plants cut 2: 15 p.m.

Time of standing, minutes	0	70	145
P_H value	5.920	5.973	6.050

(b) Plants cut 8: 20 a.m.

Time of standing, minutes	0	95
P_H value	5.802	5.907

(c) Plants cut 8: 30 a.m.

Time of standing, minutes	0	90
P_H value	5.711	5.788

(d)

Time of standing, minutes	0	100
P_H value	5.935	5.987

(e)

Time of standing, minutes	0	105
P_H value	5.790	5.956

(f)

Time of standing, minutes	0	170
P_H value	5.900	5.793

Effect of diurnal changes on the acidity of the juice from cowpeas

The acidity changes in the juice from the leaves, stems, and roots of cowpeas were followed for a period of 24 hours. The cowpeas were grown in the greenhouse in a large cylinder containing a slightly acid sand soil and were 32 days old when the determinations were made. Inoculation was insured by adding to the soil a culture of the proper legume bacteria. Determinations of acidity of the leaves and stems were made alternately every two hours, starting at 6.30 a.m. The acidity of the roots was determined but four times during the 24-hour period. The results of these determinations are given in table 3.

TABLE 3

Diurnal changes in acidity expressed in P_H of juice from leaves, stems, and roots of cowpeas. Determinations started 6:30 a.m., July 25, 1919

TIME OF DETERMINATION	LEAVES	STEMS	ROOTS
	P_H	P_H	P_H
6:46 a.m	5.400		
7:38 a.m.		5.047	
8:32 a.m	5.417		
9:24 a.m.		5.076	
10:04 a.m.			5.834
10:37 a.m.	5.276		
11:26 a.m.		5.166	
12:42 p.m.	5.369		
1:22 p.m.		5.186	
2:47 p.m.	5.426		
3:43 p.m.		5.210	
4:41 p.m.			5.742
4:57 p.m.	5.517		
5:45 p.m.		5.319	
6:40 p.m.	5.499		
7:42 p.m.		5.294	
8:25 p.m.	5.529		
9:41 p.m.		5.323	
9:05 p.m.			5.741
10:43 p.m.	5.607		
11:23 p.m.		5.314	
12:32 a.m.	5.813		
1:37 a.m.		5.216	
2:27 a.m.	5.451		
3:56 a.m.		5.186	
3:13 a.m.			5.769
4:31 a.m.	5.451		
5:12 a.m.		5.151	

In figure 1 these results are plotted as curve with the time of day as abscissas and the P_H values as ordinates. These curves show a periodicity in P_H for the different parts of the plant.

It is to be noted that in the leaves the highest acidity occurs in the morning, from which time on the acidity decreases. This is in accord with the results of previous investigators to which reference has been made. In case of the stems, the curve shows the same general trend as in case of the leaves.

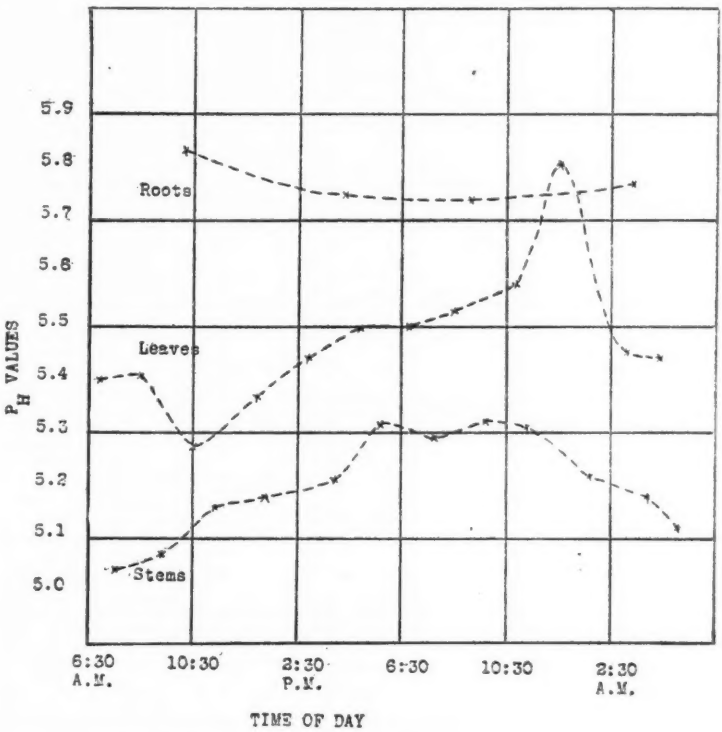

FIG. 1. CURVES SHOWING DIURNAL CHANGES IN ACIDITY OF LEAVES, STEMS, AND ROOTS OF COWPEAS

Acidity determinations started 6:30 a.m., July 25, 1919

The acidity gradually decreases during the day, reaching its lowest point about 9.30 p.m. from which time on it gradually increases. The curve for the roots indicates a reverse in acidity changes from those of the leaves and stems. The time of highest acidity in the roots appears to occur in general during the day. This may possibly be due to the faster removal of the bases from the roots during the day-time because of a greater transpiration stream.

Effect of soil on acidity of plant juice

Crops of oats, buckwheat, soybeans, and cowpeas were grown on a strongly acid (30) sand soil receiving different treatments and the P_H values of the juices of the plants were determined at different stages of their growth.

The plants were grown in the greenhouse in pots containing 11,500 gm. of Plainfield sand. Each pot received 2 gm. of K_2HPO_4 added in solution and mixed thoroughly through the soil. One-half of the pots received in each 36.8 gm. of finely ground limestone, which was at the rate of about 4 tons per acre, and was mixed into the soil at the same time with the K_2HPO_4.

For growing the plants which were used in their early stages of growth, pots containing one-half the amounts of soil stated were used. Fifty-six pots in all were used for the four kinds of crops. The jars were seeded on June 19. In order to determine what the natural or normal acidities of seedlings of these plants might be when unaffected by soil nutrients, seeds of these plants were at this same time germinated in pure white quartz sand without the addition of nutrients.

TABLE 4

Acidity of stems and leaves of seedlings grown in quartz sand without nutrients. Seeded June 19, 1919

DATE OF DETERMINATION	P_H VALUES OF PLANTS			
	Oats	Buckwheat	Soybeans	Cowpeas
June 27				5.724
July 1	5.576	4.332	5.965	
July 4	5.534			5.648
July 8			6.119	

On June 23 the soybeans and cowpeas were inoculated with the proper legume bacteria and to each of the large jars of oats and buckwheat 0.5 gm. NH_4NO_3 was added in solution. The small jars received 0.25 gm. each. On July 13 a second application of K_2HPO_4 of 0.5 gm. in solution was added to each of the large jars.

Differences in the size of the plants due to the favorable action of the lime were noticeable in case of oats and buckwheat on June 26, which difference had disappeared by July 13. Soybeans responded to lime and a distinct difference between the limed and unlimed jars was maintained throughout the growing period. The cowpeas showed no increase in growth due to lime, but the plants of the limed soil were darker green in color. In fact lime produced a darker green color in all the plants. At the time of the last determinations of the acidity of the plants, the oats and buckwheat were in the flowering stage but the soybeans and cowpeas had not yet reached that stage.

In table 4 the acidities of the juice of the seedlings grown in white quartz sand are given. In case of the soybeans and cowpeas the cotyledons were discarded.

In tables 5, 6, 7, and 8 are given, respectively, the acidities at different stages of growth of oats, buckwheat, soybeans, and cowpeas grown on unlimed and limed Plainfield sand. Acidities in different parts of the plants also are given.

TABLE 5

Acidity of the oat plant at different stages of growth and in its different parts. Oats seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
July 1	Whole top	5.812	5.783
July 4	Whole top	6.009	5.859
July 10	Whole top	5.938	5.896
July 14	Whole top	6.102	5.955
July 22	Whole top	5.843	5.518
July 28	Leaves	5.968	5.722
July 28	Stems	5.954	5.854
July 28	Roots	6.197	6.368

The results in table 5 on oats show that the leaves and stems of the limed plants were more acid than those of the unlimed plants. The roots, however, were more acid in the case of the unlimed plants.

TABLE 6

Acidity of the buckwheat plant at different stages of growth and in its different parts. Buckwheat seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
July 1	Whole top	4.832	5.429
July 8	Whole top	4.696	4.878
July 10	Whole top	5.003	4.823
July 13	Whole top	5.170	5.219
July 18	Whole top	4.949	5.205
July 20	Leaves	5.362	5.931
July 20	Stems	5.153	4.968
July 28	Leaves	5.284	5.755
July 28	Stems	4.237	4.378

In table 6 the acidity of buckwheat plants is shown in general to decrease where lime was added. Only in two cases did the limed plants show a greater acidity. The acidity of the roots was not determined because of insufficient material.

In table 7 the results for soybeans show that where the whole tops or leaves are compared, the limed plants had the greater acidity. The stems of the limed plants were less acid in two of three cases. The roots of the limed plants as in the case of oats were also less acid.

TABLE 7

Acidity of the soybean plant at different stages of growth and in its different parts. Soybeans seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
July 1	Whole top	5.970	5.947
July 8	Whole top	6.246	6.149
July 13	Whole top	6.408	6.258
July 14	Stems	6.092	6.179
July 22	Leaves	6.533	6.276
July 22	Stems	5.981	5.795
July 28	Leaves	6.272	6.247
July 28	Stems	5.827	5.843
July 28	Roots	5.947	5.997

Table 8 gives the results with cowpeas. The leaves of the limed plants are the more acid as is the case of the leaves of the limed soybeans and oats. With the tops and leaves together the limed plants are less acid, with one exception. It is to be noted again that the roots of the limed plants are less acid, as were those of oats and soybeans.

TABLE 8

Acidity of the cowpea plant at different stages of growth and in its different parts. Cowpeas seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
June 27	Whole top	5.685	5.765
June 30	Whole top	5.518	5.531
July 8	Whole top	5.471	5.450
July 12	Whole top	5.659	5.731
July 22	Leaves	5.811	5.630
July 22	Stems	5.431	5.348
July 27	Leaves	5.703	5.552
July 27	Stems	5.552	5.652
July 27	Roots	5.785	5.833

In a study and comparison of the data from the four crops, several unexpected results occur. There is, however, a direct correlation between soil and plant-root acidities. The acidity of the buckwheat roots could not be determined because the amount of material was too limited. However, it seems safe to conclude that the roots of buckwheat plants grown on limed soil were less acid, as was the case with the stems and in most cases with the leaves. This correlation between plant-root acidity and soil acidity is an important one, and is what normally would be expected. It indicates that an acid condition of the soil may limit the supply of bases to an extent sufficient

to raise the plant-root acidity. The rise in plant-root acidity undoubtedly limits the supply of bases needed by the growing stems and leaves. The rise in root acidity may also act unfavorably on the symbiotic bacteria which live in nodules attached to the roots of the legumes. This would be in direct correlation with the results of Fred and Loomis (9) and Fred and Davenport (8) which show that legume bacteria have a rather narrow optimum range of acidity.

In the case of the leaves of the different plants, with the exception of buckwheat, the results are the reverse of what they are with the roots. From these results the effect of lime on the acidity of leaves may be explained as follows. Since the effect of lime is in general to produce healthier and more vigorous plants, it is possible that in some cases these plants may have a higher acidity than those not receiving lime. In healthier and more vigorous plants metabolic processes are taking place more rapidly and if the production of acids is a measure of protein or carbohydrate metabolism, the larger amounts of acids in the leaves can be explained, even though the supply of bases in the roots of limed plants is greater. However, to establish this point more

TABLE 9

A comparison of effects of NH_4NO_3 *and* $NaNO_3$ *on the acidity of oats grown on unlimed and limed acid Plainfield sand. Oats seeded July 3, 1919*

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES			
		NH_4NO_3		$NaNO_3$	
		Unlimed	Limed	Unlimed	Limed
July 16	Whole top	5.877	5.857	5.829	5.777
July 19	Whole top	5.910	5.926	5.950	5.881

fully it would be necessary to correlate by plant analysis the protein and carbohydrate content, and perhaps ash content, with the plant acidity. Much information could probably also be obtained from comparisons of the diurnal changes in acidity of limed and unlimed plants.

To the plant cultures with Plainfield sand, NH_4NO_3 was added in order to insure an ample supply of nitrogen. Undoubtedly in the unlimed soil the ammonium part of the salt did not change to nitrates as rapidly as in the limed soil and hence there was the possibility that this ammonium might lessen the acidity of the plants. In order to get some information regarding this, oats were grown on Plainfield sand supplied with the same treatment as previously given, with the exception that in one set the nitrogen was supplied as NH_4NO_3 and in the other as $NaNO_3$. The results are given in table 9. The acidity of the plants was less in the case of the unlimed soil when either sodium or ammonium nitrate was used, with one exception. With the exception of one case the plants receiving ammonium nitrate were less acid than those receiving sodium nitrate. These results indicate that fertilizers may have an appreciable effect on plant acidity.

In table 10 a comparison of the effects of sodium and ammonium nitrates on the acidity of oat seedlings grown on quartz sand without nutrients is given. In this case the plants grown with $NaNO_3$ were less acid.

TABLE 10

A comparison of the effects of NH_4NO_3 *and* $NaNO_3$ *on acidity of 7-day-old oat seedlings grown on quartz sand*

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		NH_4NO_3	$NaNO_3$
July 21	Whole top	5.658	5.750

SUMMARY

Views concerning the formation of acids within the plant and factors affecting their accumulation have been briefly reviewed. These views conflict in many cases. One of the most plausible views concerning acid formation is held by Richards (25). He supposes that the living protoplasm forms with the carbohydrates unstable unions which by intramolecular respiration processes break down, giving organic acids as by-products. Under good conditions of oxidation these would be largely broken down into water and carbon dioxide. Incomplete oxidation of carbohydrates existing in one form or another thus seems to be one of the main sources of acids other than carbon dioxide. The relation of protein metabolism to acid formation is not definitely known but undoubtedly some acids at least may be formed in these processes. Acids accumulate at night and are destroyed in the daytime, hence it seems reasonable to believe that high illumination and high temperatures bring about rapid destruction of plant acids.

In making determinations of acidity or hydrogen-ion concentration of plant juices it is very important to take into consideration the following. Expressed plant juice usually becomes more acid on standing. Plants when cut and allowed to stand some time before the juice is expressed usually become more alkaline. Where a comparison is to be made between two sets of plants, they should all be cut at the same time and the determinations made as quickly as possible.

The diurnal changes in the acidities of the leaves, stems, and roots of the cowpea were followed during a 24-hour period. In conformity with the work of other investigators using different methods, the acidity of the leaves and stems was found to be highest in the morning and decreased during the day. The acidity of the roots was found to be highest during the day. The higher acidity of the roots in the day may possibly be due to the faster rate of movement of bases from the roots up into the plant during the day because of a greater transpiration stream at this time. The decreased acidity of the leaves and stems in the day is attributed to the destruction of the accumulated acids by influences as increased light and higher temperatures. Undoubtedly diurnal changes in plant processes also are factors.

Acidity measurements were made of oats, buckwheat, soybeans, and cowpeas at different stages of their growth and in their different parts. Plainfield acid sand, part of which received sufficient lime to neutralize the acid present, was used. Many unexpected results were obtained. With the exception of buckwheat the tops of the limed plants were usually more acid. There is thus an indication that in some cases lime, by stimulating the natural life and growth processes of the plant, causes a rise in the production of acids and hence a higher acidity within the leaves where the most active processes take place.

The acidity of the roots of the plants appears to correlate with the acidity of the soil. The relation of soil acidity and liming to plant growth and the symbiotic legume bacteria is thus indicated, since the roots are the source of the lime needed by the other parts of the plant for neutralizing acids and other purposes.

The acidity of the tops of oats grown on unlimed soil is less than in limed soil when either $NaNO_3$ of NH_4NO_3 is used as a source of nitrogen, with one exception. With pure quartz the use of $NaNO_3$ produced plants of less acidity than the use of NH_4NO_3. With Plainfield sand soil the reverse was usually the case. The form of fertilizer thus appears to affect the acidity of plants to some extent.

The investigation reported has been largely on methods and hence is of a preliminary nature. Further investigations are needed before definite conclusions can be drawn regarding the influence of soil acidity and fertilizers on the acidity and metabolic processes of plants. The influences of the soil upon the acidity of the plants could undoubtedly be made clearer, if the diurnal changes of the acidities of the plants were followed in all cases. Plant analysis for protein, carbohydrate, and ash content might also show something of the relation between acid formation and the formation or destruction of these classes of compounds, as well as their relation to the ash and especially lime content.

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THE INFLUENCE OF LIME ON THE NITRIFICATION OF BARNYARD MANURE-NITROGEN IN ARABLE SOIL

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INTRODUCTION

Experiments made by Barthel and others have shown that the part of the nitrogen in solid manure that is first nitrified in the soil is the ammonia-nitrogen. This is only what is to be expected. However, the experiments showed further, not merely that the higher-nitrogen compounds, apparently, are not nitrified until after the lapse of a very long time, but that even the ammonia-nitrogen present from the outset is not in its entirety converted into saltpetre during the first year of growth. Moreover, it was found that in well-tilled arable soil with a decidedly acid reaction, nitrification can take place with as much energy, at least, as in a soil of neutral reaction. It is true that these experiments were not made under conditions exactly similar to those prevailing in agricultural practice; but the circumstances were extremely favorable for the rapid and energetic operation of the nitrification process.

Thus previous experiments had shown that even in conditions extremely favorable to nitrification only a part, larger or smaller, of the ammonia nitrogen of solid manure is nitrified during the first year of growth; but it still remained to be ascertained whether the fertilizing action of the stable manure could be enhanced by any of the artificial fertilizers which are within the reach of the farmer.

The first factor considered was lime. From many points of view lime is a very valuable fertilizer, mainly because of its basic properties and its consequent capacity for regulating the hydrogen-ion concentration in the soil in a manner favorable to bacterial life. However, few experiments had hitherto been made with the direct object of ascertaining the action of lime on the nitrification of stable manure, and as these were by no means decisive, it was considered well worth while to take up this question for investigation.

The method principally employed was that adopted in our previous experiments on nitrification. Briefly, it was as follows. The different ingredients were first carefully blended with the soil which was then placed in glass jars holding 5 kgm. each. The soil was well packed down by knocking each jar on the floor a certain number of times, and each jar was sealed with a cork, in the middle of which was a short glass tube containing cotton-wool. By this

simple procedure the evaporation is practically reduced to nil, even if the soil is kept for a very long time, while the air has practically unimpeded access to the soil. During the experiments the jars were kept in the laboratory at a temperature of 15 to 25°C. according to the season of the year. At regular intervals samples were analyzed for their content of nitrate-nitrogen, ammonia-nitrogen and moisture.

The nitrate-nitrogen was determined colorimetrically in accordance with the phenol-sulfuric acid method. The content of moisture in the soil in our experiments remained constantly between 15 and 20 per cent, the most favorable percentage for the formation of nitrate.

A number of experiments were made in the determination of the ammonia content of the soil, and are recorded below.

DETERMINATION OF THE PERCENTAGE OF AMMONIA IN THE SOIL

As McBeth (5) has shown, ammonia which is added to the soil either in a free state, or in the form of ammonium salts, cannot be quantitatively recovered by any of the usual methods for the determination of ammonia in soil. McBeth tested the methods of both Boussingault (direct distillation with MgO) and Schlösing (extraction with HCl and the distillation of the extract with KOH) and found them to be approximately of equal value. When 50 mgm. of ammonia-nitrogen were added to 100 gm. of earth, the nitrogen losses in the case of two soils examined by McBeth amounted to 48 and 80 per cent. By direct distillation with KOH or by boiling with HCl for 4 hours and distilling the extract with KOH, the losses could be reduced considerably. However, neither of these methods seems to be desirable, as in both cases the splitting of ammonia out of the organic nitrogenous elements of the soil is to be feared. McBeth found also that the capacity of the soil for binding ammonia, with the exception of humus soils, increases directly with the depth below the surface.

The results of some experiments on three different soils are set forth in table 1. The stated admixtures of ammonia were supplied in the form of a solution of sulfate of ammonia (25 cc.), which was allowed to soak in the soil for at least 30 minutes before the determinations of ammonia were made (in accordance with the Boussingault method). The original content of ammonia in the soil was, of course, determined at the same time. The distillate amounted to 350 to 400 cc. All the analysis figures in this and the following tables are the average of two determinations. Soil III was a humus soil.

These experiments thus confirm McBeth's results, even though the losses here are much smaller. Even in this latter respect our results tally with those of McBeth in principle seeing that in the case of humus soils the losses did not increase with the depth beneath the surface.

There is thus reason to doubt the correctness of the ammonia figures obtained for original soils with the use of the ordinary method of distillation with MgO. A method for the determination of ammonia cannot in fact be regarded

as reliable unless the results of the analysis show the original ammonia content in the soil plus the ammonia added.

Baragiola and Schuppli (1) maintain that they have solved this problem. After adding 100 cc. of water to 50–100 gm. of soil, they distilled off to dryness with MgO in a vacuum of 15 mm. Hg. at 35°C. The cupellation was facilitated by an ammonia-free current of air, which was supplied through a capillary tube. In all the cases investigated they recovered quantitatively the ammonia added.

TABLE 1

Determinations of ammonia in soil according to Boussingault's method

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN ADDED TO 50 GM. SOIL	AMMONIA-NITROGEN RETAINED IN PERCENTAGE OF THE AMOUNT ADDED
	cm.	*mgm.*	*per cent*
Soil I	5–15	53.8	4.39
	25–35	53.8	7.71
Soil II	5–15	53.8	7.94
	25–35	53.8	16.28
Soil III	5–15	53.8	5.19
	25–35	53.8	3.73

TABLE 2

Determination of ammonia in soils: comparison of methods of Boussingault and of Baragiola and Schuppli

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN ADDED TO 50 GM. SOIL	LOSS OF AMMONIA-NITROGEN IN PERCENTAGE OF THE AMOUNT ADDED	
			Baragiola and Schuppli method	Boussingault method
	cm.	*mgm.*	*per cent*	*per cent*
Soil I	5–15	53.8	5.21	1.56
Soil II	5–15	53.8	13.42	7.94
	25–35	53.8	27.60	16.28
Soil III	5–15	53.8	2.93	15.10

Table 2 gives the results obtained in comparative experiments with the last-mentioned method and the Boussingault method, when the soil was supplied with $(NH_4)_2SO_4$.

Thus only in one case was the vacuum method superior to the ordinary distillation. In the three other cases, on the other hand, it yielded far *inferior* values to the Boussingault method. The explanation why Baragiola and Schuppli obtained such good results with their method must be that they worked with soils which were very weak binders of ammonia. It is manifest

from both tables 1 and 2 and from the figures given by McBeth that this absorbing capacity may in fact vary considerably for different soils.

The determinations of ammonia in the soils without admixture of ammonia gave the values shown in table 3.

Baragiola and Schuppli found that the soils investigated by them were entirely free from ammonia, with the exception of a strongly manured garden soil and a dry peat soil, which contained, respectively, 1.45 and 0.15 mgm. ammonia-nitrogen per 50 gm. of soil. As is evident from table 3, the vacuum method in this case too gave low values, which are considerably surpassed by the results obtained with the Boussingault method. However, as neither of the methods quantitatively showed the ammonia added, it is not possible to decide which of them gives the more correct value for the ammonia content of the soil. It is equally possible that the Boussingault method is more capable of separating adsorbed ammonia, and that the capacity of the vacuum method in this respect is inadequate, as the increased output in accordance with the Boussingault method is due to a splitting of organic nitrogen compounds.

TABLE 3

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN IN 50 GM. SOIL	
		Baragiola and Schuppli method	Boussingault method
	cm.	*mgm.*	*mgm.*
Soil I	5–15	0.21	2.24
Soil II	5–15	0.07	3.50
	25–35	1.89	9.66
Soil III	5–15	0.07	8.40

There is certainly no reason to prefer the vacuum method to the Boussingault method, especially as the latter seems to release more adsorbed ammonia (table 2).

We have also tested the following method, elaborated by Potter and Snyder (8) for the determination of ammonia nitrogen in soil, a method for which entire reliability is claimed. A retort is charged with 25 gm. of soil, 50 cc. of ammonia-free water, a few drops of some heavy mineral oil (in order to prevent foaming) and about 2 gm. Na_2CO_3. The retort is in connection with a flask containing N/10 H_2SO_4 and an air pump. Ammonia-free air is sucked through the apparatus at a speed of 250 liters an hour.

The experiments were made on soil II from 5–15, 15–25 and 25–35 cm. depths. At the same time determinations were made according to the Boussingault method. The results are set forth in table 4.

Twenty-five grams of each of the soils contained 0.07, 0.0 and 0.0 mgm. of ammonia nitrogen, respectively, in accordance with the Potter and Snyder method, and 2.82, 3.29, and 2.73 mgm. according to the Boussingault method.

As is evident from the table, the Potter and Snyder method also, in spite of the fact that the prescribed airing period was increased to 20 hours, did not come up to promise, but on the contrary gave decidedly inferior results to the Boussingault method.

Some attempts were made also to determine the content of ammonia in soil in accordance with the Steinkopf (9) method—distillation with water vapour in a vacuum (with MgO). In this case a more effective liberation of the adsorbed ammonia was possibly to be expected without risking splitting-off of organic nitrogen compounds than with the Baragiola and Schuppli vacuum

TABLE 4

Determination of ammonia in soils; comparison of methods of Potter and Snyder and of Boussingault

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN ADDED TO 25 GM. SOIL	AMMONIA-NITROGEN RETAINED IN PERCENTAGE OF THE AMOUNT ADDED	
			Potter and Snyder method	Boussingault method
	cm.	*mgm.*	*per cent*	*per cent*
Soil II	5–15	21.56	19.48	4.22
	15–25	21.56	17.21	12.01
	25–35	27.02	24.48	20.23

TABLE 5

Determination of ammonia nitrogen in soil II: Comparison of methods of Steinkopf, of Baragiola and Schuppli and of Boussingault

METHOD	AMMONIA-NITROGEN FOUND IN		LOSS IN PERCENTAGE OF NITROGEN ADDED
	50 gm. soil	50 gm. soil + 53.76 mgm. ammonia-nitrogen	
	mgm.	*mgm.*	*per cent*
Steinkopf	0.14	50.82	5.73*
Baragiola and Schuppli	0.07	44.59	17.18
Boussingault	2.24	51.66	8.07

* After the second distillate. After the first the loss was 11.72 per cent.

method. An experiment with the Steinkopf method on soil II, which had proved to possess the greatest capacity for binding ammonia, gave the values shown in table 5. In these experiments the pressure was 40 mm. Hg and the temperature of the water bath 40°C. Two distillates were taken, each of 450 cc. For the purpose of comparison, the corresponding figures for parallel determinations in accordance with the Baragiola and Schuppli and the Boussingault methods are included.

With regard to the separation of ammonia added, the Baragiola and Schuppli method was evidently far inferior to that of Steinkopf. The latter vacuum method has proved to be about as effective as the Boussingault method.

It is considered (3, 6, 7) that the capacity of the soil for adsorbing ammonia, partly at any rate, is to be ascribed to zeolites. The latter contain Na, Ca or Mg, which on treatment with a diluted solution of a salt of ammonia are replaced by NH_4. If, on the other hand, the equation is NH_4—zeolite, the opposite is the case. In order to test the theory, the following experiment was made.

Fifty grams of soil II containing 5.04 mgm. ammonia-nitrogen in accordance with the Boussingault method, were admixed with 300 cc. of $(NH_4)_2SO_4$ solution (53.76 mgm. N). After 48 hours the solution was filtered and washed with 400 cc. distilled water, and the ammonia-nitrogen of the filtrate was determined at 31.29 mgm. The residue of the filtrate was then allowed to stand with 300 cc. $MgCl_2$ solution (1 gm. $MgCl_2+6H_2O$), whereupon it was filtered and washed with distilled water. This latter filtrate contained 9.03 mgm. ammonia nitrogen and in the undissolved residue 12.32 mgm. was found. The nitrogen loss was consequently 11.46 per cent of the amount of nitrogen added. In a corresponding parallel determination by the Boussingault method (distillation of 50 gm. soil + 53.76 mgm. ammonia-nitrogen after 48 hours) the loss was 7.42 per cent. In this instance, therefore, nothing had been gained by the substitution process.

It is thus manifest from all our experiments that there exists at present no method which permits of an accurate quantitative extraction of ammonia from any soil. Thus in the Boussingault method, which was employed by us in all the following experiments, it will always be necessary to reckon with some loss. However, in our experiments these losses are scarcely of any account, because the absolute losses in the soils worked with are relatively small, and also because the experiments in question are concerned with relative values.

EXPERIMENTS RELATING TO THE ACTION OF LIME ON THE NITRIFICATION OF STABLE-MANURE NITROGEN IN SOILS OF DIFFERENT REACTION

In the first experiment the soil designated above as soil II was used. This was taken from the experimental field of the Botanical Section of Experimentalfältet, and consisted of an originally stiff clay soil, now well manured, friable, and well tilled. The reaction to litmus was neutral. As the *Azotobacter* test gave a positive reaction, this soil was evidently not in need of lime. Before the soil was placed in the glass jars, it was riddled and supplied with the following carefully mixed ingredients.

Jar I No admixture (control).
Jar II 10 gm. $(NH_4)_2SO_4$ (0.2 per cent).
Jar III 10 gm. $(NH_4)_2SO_4$ + 89.5 gm. $CaCO_3$ (1 per cent CaO).
Jar IV 150 gm. 3 to 4 months old barnyard manure (3 per cent).[1]
Jar V Manure + lime in the same quantities as before.

[1] The content of total nitrogen and of ammonia-nitrogen in the manure was determined at each experiment, but these figures are not give here. In order to obtain clear results only manures whose content of ammonia-nitrogen exceeded 0.14 per cent were used.

The manure did not contain any liquid, as the latter had been collected separately in a special manure-water well.

Both the nitrate-nitrogen and the ammonia-nitrogen in this experiment, as in all the following, are expressed in terms of milligrams per kilogram of moist earth.

The results of this first experiment are shown in table 6. The content of moisture in the soil is not given in each separate case. During the entire course of the experiment it was about 20 per cent in the jars which did not contain any manure, and in the manure jars 21 to 22 per cent.

In examining these figures it should be borne in mind that one must not look for precise agreement even where theoretically it might be expected, as, for example, in the content of nitrate in all the jars at the beginning of the experiment, or the content of ammonia at the beginning in jars II and III, or IV and V, respectively. Such divergencies in work of this kind are inevitable, partly because of the impossibility of mixing with perfect homogeneity the components added to the soil, and partly because of the difficulty of obtaining actual average samples from the jars. In spite of such discrepancies, however, various conclusions may be drawn from the experiment.

First, the content of nitrate in the soil is unusually high from the very outset, so high that any greater nitrification in the control soil is scarcely noticeable. This can be explained, at any rate in part, by the fact that the experimental soil, being originally rather moist, had first to be spread out to dry at ordinary temperature before it could be put into the glass jars. During this drying, however, a very intense nitrification takes place.

It will further be found that the admixture of lime has an extremely stimulating effect on the nitrification of the ammonium sulfate. It is precisely for the purpose of establishing this effect that the series with ammonium sulfate and that with ammonium sulfate plus lime have been included in all our experiments.

The nitrification of the manure-nitrogen also was rather marked. In previous experiments (2) it has been proved that it is only the ammonia-nitrogen of the manure that is nitrified. This is confirmed also by the present and following experiments, which show that the nitrate-nitrogen formed does not exceed the ammonia-nitrogen present from the outset in the mixture of soil and manure, deducting the normal content of ammonia-nitrogen in the soil. The soil always contains a residue of 20 to 50 mgm. of ammonia-nitrogen per kilogram which does not allow of nitrification. This may conceivably be due to the fact that this nitrogen originally did not consist of ammonia-nitrogen proper, but was produced after distilling with MgO by the splitting-off of higher compounds of nitrogen.

Proceeding to the main objective of the experiments, namely, to the possible action of the lime on the nitrification of the stable-manure nitrogen, table 6 shows clearly that in this experiment no stimulating action of the lime can be found. On the contrary, the average of the nitrate-nitrogen for the entire series is lower for the manure plus lime than for the manure alone.

TABLE 6

Influence of lime on nitrification—First experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE-NITROGEN AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL											
			0 days	7 days	14 days	21 days	28 days	35 days	41 days	49 days	56 days	146 days	236 days	334 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	48.0	46.0	48.0	52.0	52.0	52.0	48.0	52.0	52.0	56.0	56.0	72.0
		Ammonia-nitrogen	47.6	44.8	44.8	44.8	47.6	42.0	—	44.8	36.4	25.2	50.4	39.2
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	32.0	68.0	96.0	170.0	210.0	280.0	200.0	220.0	200.0	240.0	290.0	100.0
		Ammonia-nitrogen	414.4	411.6	310.8	252.0	240.8	196.0	140.0	120.4	95.2	28.0	42.0	42.0
III	0.2 per cent $(NH_4)_2SO_4$ + 1 per cent CaO	Nitrate-nitrogen	28.0	60.0	128.0	200.0	260.0	280.0	340.0	360.0	280.0	290.0	280.0	340.0
		Ammonia-nitrogen	456.4	436.8	386.4	285.6	201.6	114.8	44.8	42.0	36.4	28.0	—	42.0
IV	3 per cent manure	Nitrate-nitrogen	40.0	60.0	56.0	68.0	76.0	68.0	80.0	80.0	80.0	100.0	88.0	96.0
		Ammonia-nitrogen	84.0	47.6	44.8	47.6	50.4	50.4	42.0	50.4	39.2	28.0	47.6	42.0
V	3 per cent manure + 1 per cent CaO	Nitrate-nitrogen	44.0	60.0	58.0	80.0	76.0	72.0	60.0	68.0	72.0	68.0	76.0	88.0
		Ammonia-nitrogen	70.0	47.6	50.4	47.6	53.2	50.4	42.0	47.6	42.0	28.0	44.8	47.6

Stephenson (10), who examined the effect of organic matter on the production of nitrate and ammonia nitrogen in different soils, in this connection obtained similar results. He says that "the sum of the nitrates and ammonia is greater in most cases on the unlimed than on the limed pots where organic treatments are given."

In the next experiment the proportion of lime to manure was increased, the ratio being 2 per cent of CaO to 1 per cent of manure, as compared with 1 and 3 in the preceding experiment. In other respects, both the experimental soil and the amount of ammonium sulfate added were the same as before. The water content of the soil was about 22 per cent. The results of this experiment are shown by table 7.

In view of the greatly reduced admixture of manure in this experiment, one could hardly expect to obtain very high nitrate figures for jar IV. It is, however, possible to show a more powerful nitrification here than in the control jar. The admixture of lime in this experiment also gave a negative result, since the amount of nitrate in jar V is somewhat lower than in jar IV, and as a matter of fact does not exceed that of the control jar. Here it seems to be practically certain that the addition of lime impeded the nitrification of the nitrogen of the barnyard-manure.

In this experiment and those that follow, a control series was conducted with the same soil and the same admixtures, but at a different time. The control series naturally did not give the same absolute figures for the nitrate and the ammonia nitrogen as the corresponding series, but the relative proportions were invariably the same. The results of the control series are not given here, as this would unnecessarily congest the tabular material, but the fact that they were always carried out, and invariably agreed well with the experiments described is strong proof of the reliability of the results.

In the succeeding experiment the same amount of lime was retained, but the supply of manure was increased to 5 per cent. Such an amount of manure, of course, is much too large for practical purposes, being equivalent to no less than 150,000 kgm. per hectare, while the amounts previously used are equivalent to 90,000 and 30,000 kgm. per hectare. However, in these experiments the admixtures were greatly varied because the aim was to ascertain whether the relative amounts of lime and manure had any influence at all on the results. The soil used in this experiment was the one designated as soil I in the ammonia experiments. It is a well-cultivated clay loam (ancient sea bottom) from Experimentalfältet. The reaction was neutral and the *Azotobacter* test gave a positive result. The soil has long been in a high state of fertility.

From table 8 it can be seen that here also the lime considerably impeded the nitrification of the stable-manure nitrogen. The original content of nitrate in the soil was very low. The ammonium sulfate added was not nitrified as much as in the preceding experiments, but when lime was added, this nitrification, on the other hand, was much greater than in the experiments with soil II. It will be found in this, as in preceding experiments, that in

TABLE 7

Influence of lime on nitrification—Second experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL								
			0 days	14 days	21 days	28 days	35 days	42 days	138 days	266 days	356 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	32.0	38.0	36.0	36.0	39.0	48.0	56.0	52.0	44.0
		Ammonia-nitrogen	56.0	50.4	64.4	58.8	61.6	56.0	33.6	44.8	47.6
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	26.0	112.0	200.0	220.0	240.0	300.0	360.0	230.0	220.0
		Ammonia-nitrogen	425.6	291.2	263.2	—	207.2	154.0	39.2	44.8	39.2
III	0.2 per cent $(NH_4)_2SO_4$ + 2 per cent CaO	Nitrate-nitrogen	20.0	180.0	260.0	280.0	300.0	380.0	360.0	270.0	200.0
		Ammonia-nitrogen	383.6	170.8	64.4	56.0	53.2	53.2	36.4	44.8	42.0
IV	1 per cent manure	Nitrate-nitrogen	28.0	36.0	36.0	40.0	46.0	56.0	68.0	64.0	56.0
		Ammonia-nitrogen	56.0	58.8	44.8	58.8	53.2	56.2	36.4	50.4	53.2
V	1 per cent manure + 2 per cent CaO	Nitrate-nitrogen	26.0	36.0	36.0	36.0	40.0	48.0	56.0	56.0	60.0
		Ammonia-nitrogen	53.2	53.2	47.6	61.6	56.0	50.4	39.2	50.4	53.2

TABLE 8

Influence of lime on nitrification—Third experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL													
			0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days	77 days	84 days	91 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	1.0	3.6	4.0	5.5	6.0	8.0	8.0	8.0	8.0	10.0	8.0	10.0	12.0	10.0
		Ammonia-nitrogen	33.6	30.8	22.4	25.2	33.6	22.4	28.0	33.6	39.2	25.2	19.6	19.6	25.2	19.6
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	0.8	5.5	15.0	24.0	44.0	68.0	65.0	65.0	70.0	70.0	80.0	90.0	80.0	70.0
		Ammonia-nitrogen	—	476.0	434.0	361.2	324.8	319.2	302.4	285.6	260.4	246.4	240.8	243.6	251.2	232.4
III	0.2 per cent $(NH_4)_2SO_4$ + 2 per cent CaO	Nitrate-nitrogen	1.0	11.0	48.0	80.0	180.0	220.0	260.0	240.0	220.0	240.0	200.0	220.0	200.0	140.0
		Ammonia-nitrogen	504.0	420.0	252.8	170.8	33.6	25.2	28.0	22.4	30.8	19.6	19.6	16.8	28.0	25.2
IV	5 per cent manure	Nitrate-nitrogen	1.6	10.0	16.0	20.0	28.0	30.0	30.0	44.0	32.0	36.0	28.0	34.0	28.0	18.0
		Ammonia-nitrogen	70.0	56.0	28.0	39.2	28.0	28.0	16.8	28.0	28.0	33.6	28.0	22.4	39.2	22.4
V	5 per cent manure + 2 per cent CaO	Nitrate-nitrogen	1.2	8.0	7.5	14.0	16.0	20.0	18.0	12.0	14.0	18.0	16.0	16.0	16.0	14.0
		Ammonia-nitrogen	56.0	33.6	28.0	28.0	16.8	22.4	19.6	36.4	25.2	19.6	22.4	22.4	28.0	28.0

the case of the admixture of ammonium sulfate (especially with $(NH_4)_2SO_4$ plus CaO), a continuous diminution occurs in the sum of the nitrate and ammonia nitrogen at each sampling from the different jars. This can be explained by an assimilation brought about by microorganisms. It seems hardly conceivable that ammonia losses should arise by a purely chemical process, i.e., by the action of the lime, if one may judge by the experiments made in particular by Lemmermann and his collaborators. On the other hand, it has been found that the addition of lime markedly promotes the assimilation of ammonia and nitrate in the soil. In the jars containing manure no such reduction of the summed-up nitrate and ammonia nitrogen occurs.

All the above experiments are open to the objection that they have been made with amounts of lime which greatly exceed those which are used in practice. It is, in fact, probable that when the amount of lime added has exceeded a certain limit, a further increase can have no further effect. Such large amounts of lime were here used in order to ensure the clearest possible results. However, in order to be certain that the conditions prevailing when normal amounts of lime are added are not different in principle from those present in the experiments, soil II was used for another experiment, in which lime was added at the rates of 0.2 and 0.1 per cent, being equivalent to 6,000 and 3,000 kgm. CaO per hectare. Manure was used at the rate of 2 per cent, being equivalent to 60,000 kgm. per hectare. The results obtained are given in table 9.

The figures of this table show clearly, as was to be expected, that the small amounts of lime had no greater stimulating effect on the nitrification of the stable manure than the larger. But, on the other hand, no "impeding" action by lime could here be established, as occurred with the larger applications, since nitrification evidently proceeded quite uniformly in jars IV, V, and VII.

All the experiments hitherto described have been made with neutral soils, which, according to the *Azotobacter* test, were not in need of lime. It might reasonably be argued that the cause of the negative result of the addition of lime to these soils in regard to its action on the nitrification of the manure nitrogen is simply the fact that the lime is unable to react on a soil which already contains, from a biological point of view, a sufficient amount of lime.

It is evident, nevertheless, that the lime had a certain action in our experiments, first, because the jars which, in addition to manure, contained larger quantities of lime, showed throughout an inferior nitrification to those which contained simply manure; and, second, because the lime, even in these soils which were not in need of lime, always showed a strong capacity for stimulating the nitrification of ammonium sulfate. This action is still quite evident with an addition of 0.2 per cent CaO, whereas it is hardly perceptible at 0.1 per cent.

It was conceivable that in manifestly acid soils the lime might have another effect on the nitrification of the manure-nitrogen than had been ascertained to be the case in the neutral soils hitherto used. Similar experiments were therefore conducted with an acid-reacting soil, a clay soil from Ensta in upland.

TABLE 9

Influence of lime on nitrification—Fourth experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL													
			0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days	77 days	84 days	98 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	5.5	6.0	7.5	8.0	8.5	9.0	10.0	10.5	11.0	11.0	10.5	12.0	12.0	12.5
		Ammonia-nitrogen	36.4	47.6	42.0	39.2	30.8	47.6	50.4	44.8	39.2	22.4	47.6	47.6	36.4	39.2
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	5.0	30.0	65.0	100.0	100.0	110.0	130.0	160.0	180.0	190.0	210.0	210.0	220.0	220.0
		Ammonia-nitrogen	341.6	403.2	302.4	288.4	252.0	193.2	128.8	84.0	58.8	58.8	47.6	42.0	36.4	39.2
III	0.2 per cent $(NH_4)_2SO_4$ + 0.2 per cent CaO	Nitrate-nitrogen	5.5	28.0	70.0	105.0	110.0	150.0	190.0	180.0	140.0	190.0	230.0	220.0	220.0	210.0
		Ammonia-nitrogen	394.8	375.2	296.8	238.0	182.0	112.0	39.2	50.4	42.0	47.6	47.6	36.4	33.6	44.8
IV	2 per cent manure	Nitrate-nitrogen	6.0	11.5	15.0	14.0	15.0	15.0	17.0	17.0	16.0	17.0	17.0	21.0	21.0	22.0
		Ammonia-nitrogen	47.6	36.4	42.0	47.6	44.8	50.4	33.6	44.8	44.8	36.4	42.0	47.6	39.2	39.2
V	2 per cent manure + 0.2 per cent CaO	Nitrate-nitrogen	6.0	11.5	13.0	14.0	15.0	16.0	17.0	17.0	16.0	17.0	17.0	19.0	21.0	21.0
		Ammonia-nitrogen	50.4	39.2	50.4	42.0	44.8	50.4	33.6	47.6	47.6	42.0	44.8	42.0	44.8	42.0
VI	0.2 per cent $(NH_4)_2SO_4$ + 0.2 per cent CaO	Nitrate-nitrogen	5.0	23.0	60.0	80.0	85.0	130.0	150.0	170.0	160.0	200.0	210.0	210.0	230.0	230.0
		Ammonia-nitrogen	383.6	350.0	308.0	263.2	235.2	173.6	114.8	61.6	53.2	44.8	50.4	50.4	47.6	42.0
VII	2 per cent manure + 0.1 per cent CaO	Nitrate-nitrogen	6.5	12.0	15.0	14.0	15.0	15.0	18.0	17.0	17.0	14.0	17.0	20.0	21.0	21.0
		Ammonia-nitrogen	64.4	50.4	44.8	47.6	58.8	58.8	50.4	53.2	53.2	47.6	50.4	56.0	47.6	50.4
VIII	0.2 per cent CaO	Nitrate-nitrogen	5.0	5.0	7.0	8.0	8.0	8.5	9.5	9.0	9.0	8.0	9.5	10.5	10.5	12.0
		Ammonia-nitrogen	50.4	44.8	56.0	—	58.8	58.8	67.2	53.2	53.2	56.0	53.2	56.0	47.6	50.4

TABLE 10

Influence of lime on nitrification—Fifth experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL												
			0 days	8 days	16 days	22 days	30 days	36 days	44 days	58 days	76 days	97 days	113 days	133 days	151 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	1.6	3.6	4.4	5.0	5.6	7.5	8.5	10.0	10.0	13.0	12.0	16.0	17.0
		Ammonia-nitrogen	33.6	33.6	30.8	28.0	25.2	28.0	19.6	28.0	28.0	39.2	44.8	25.2	42.0
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	1.2	12.0	11.0	23.0	30.0	32.0	44.0	48.0	40.0	50.0	44.0	48.0	54.0
		Ammonia-nitrogen	498.4	436.8	462.0	406.0	389.2	425.6	389.2	366.8	352.8	366.8	327.6	344.4	344.4
III	0.2 per cent $(NH_4)_2SO_4$ + 2 per cent C_aO	Nitrate-nitrogen	1.4	17.0	40.0	105.0	130.0	200.0	190.0	190.0	160.0	170.0	150.0	160.0	160.0
		Ammonia-nitrogen	—	422.8	394.8	308.0	159.6	58.8	39.2	30.8	30.8	36.4	42.0	25.2	42.0
IV	4 per cent manure	Nitrate-nitrogen	1.3	22.0	24.0	32.0	36.0	44.0	40.0	48.0	46.0	48.0	42.0	48.0	80.0
		Ammonia-nitrogen	70.0	47.6	33.6	44.8	30.8	28.0	25.2	25.2	28.0	42.0	42.0	25.2	—
V	4 per cent manure + 2 per cent CaO	Nitrate-nitrogen	1.4	23.0	20.0	28.0	32.0	32.0	32.0	40.0	38.0	44.0	40.0	44.0	65.0
		Ammonia-nitrogen	70.0	36.4	36.4	33.6	28.0	30.8	33.6	28.0	33.6	42.0	42.0	28.0	39.2
VI	4 per cent manure + 0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	1.0	10.0	23.0	30.0	38.0	52.0	56.0	85.0	80.0	100.0	105.0	120.0	150.0
		Ammonia-nitrogen	473.2	—	422.8	403.2	352.8	352.8	322.0	310.8	266.0	313.6	305.2	285.6	302.4
VII	4 per cent manure + 0.2 per cent $(NH_4)_2SO_4$ + 2 per cent CaO	Nitrate-nitrogen	1.0	15.0	56.0	105.0	140.0	210.0	160.0	190.0	150.0	180.0	190.0	210.0	230.0
		Ammonia-nitrogen	523.6	422.8	344.4	176.4	50.4	33.6	30.8	28.0	30.8	39.2	44.8	25.2	36.4
VIII	2 per cent CaO	Nitrate-nitrogen	1.6	5.5	4.8	7.0	8.5	7.0	9.0	11.0	10.0	15.0	16.0	21.0	25.0
		Ammonia-nitrogen	39.2	36.4	30.8	30.8	28.0	28.0	25.2	25.2	25.2	44.8	42.0	28.0	42.0

The same soil had previously been used in our experiments on the nitrification of stable manure in soils of different reaction.

This soil is in a high state of cultivation. In the qualitative test of its acidity in accordance with the Truog method, it was shown to be of "medium acidity." The reaction to litmus was evidently acid. The *Azotobacter* test gave a negative result.

In the execution of the experiment exactly the same procedure as in the foregoing was adopted. The admixture of lime was 2 per cent and the amount of stable manure was 4 per cent of the weight of the soil. As with the preceding soils, two exactly similar series of experiments were made at different times. As in this case also the results of the two series agreed with one another very closely, the results of only one series are given (table 10).

The interesting facts shown here with regard to the nitrification in the Ensta soil without admixture, in the soil with $(NH_4)_2SO_4$ alone, and in the soil with manure only, have been discussed in a previous paper (2) so are not taken up here. It need only be pointed out that the acid reaction of the soil did not stand in the way of a strong nitrification, either in the soil without admixture, or in the soil with manure, whereas in the soil with $(NH_4)_2SO_4$ the latter was nitrified very poorly, even more poorly than the stable-manure nitrogen. This fact, which has also been pointed out by other scientists, has been explained in the above-mentioned paper as follows:

> The increase in the concentration of hydrogen-ions which sets in when the ammonium sulfate begins to be decomposed in acid soils, has an impeding action on the nitrification, whereas in the case of the decomposition of the organic nitrogenous substances no such increase of the concentration of the hydrogen-ions need be considered.

As for the action of the lime, it is evident from the figures for jars IV and V that the lime had a no more favorable action on the nitrification of the stable-manure nitrogen in this acid soil than in the neutral soils. On the contrary, a manifest impeding effect is noticeable here. On the addition of lime alone to the soil a slight increase in the nitrification is certainly noticeable, but not until after the lapse of three or four months. On the other hand, as was to be expected, the lime had a decidedly favorable effect on the nitrification of the ammonium sulfate.

It should be pointed out that the quantity of lime added is far more than sufficient to neutralize the soil.

SUMMARY

All the experiments tended to show that on the addition of lime, in the form of calcium carbonate, and stable manure to cultivated soil of both neutral and acid reaction, no favorable action of the lime on the *nitrification of the stable-manure nitrogen resulted*, but on the contrary, an impeding effect, occurred in cases where the supply of lime was very large, larger than is probably ever used in practice.

It is not easy to explain the cause of this impeding action of the lime. Fred and Graul (5), who made experiments on the action of lime on the nitrification of organic substances in acid soils, found, that in the presence of casein or gelatin the addition of lime produced an enormous increase of nitrate-assimilating bacteria, and it is quite possible that the same will be the case if, as in our experiments, stable manure and lime are simultaneously supplied to the soil. If this is really the case, this impeding of nitrification does not imply any loss of nitrogen, absolutely speaking, since the nitrogen which the nitrate-assimilating baceria extracted is stored in the form of bacterial proteins and is sooner or later utilized on the death of the bacterial cells.

If, on the other hand, the impeding action of the lime on the nitrification of the stable-manure nitrogen is due to an increased development of denitrifying bacteria, a direct loss of nitrogen is naturally conceivable. At any rate these questions appear to be of no practical importance, for, as has already been pointed out, the lime does not exercise either an impeding or a stimulating action when it is supplied in quantities which occur in practice.

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QUANTITATIVE AND QUALITATIVE BACTERIAL ANALYSIS OF SOIL SAMPLES TAKEN IN FALL OF 1918

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A bacterial analysis was made of 46 soils representing 17 soil types obtained in a soil survey of Russell, Carleton and Stormont counties in Eastern Ontario. Each soil was represented by two samples, one taken from the surface to a depth of $6\frac{2}{3}$ inches, and the other a sub-surface sample taken from $6\frac{2}{3}$ to 20 inches depth. The lower subsoils were not examined for bacterial content. These soils were filled directly into cotton bags in the field where obtained and shipped to the college by freight. On arrival at the chemistry department they were emptied into large open-topped dishes for air-drying in the attic, which was clean and free from dust. The samples for bacterial analysis were taken from these open dishes a few days after their arrival and put directly into clean, dry 500-cc. Erlenmeyer flasks which were half filled and then corked and kept at a low temperature in the attic of the bacteriology department until December 15, when the analyses were begun.

The purpose of the analysis was to determine the relative bacterial and mold content of the different soil samples. Three culture media were used as follows:

1. *Albumen Synthetic Agar* (A) (P. E. Brown) for obtaining the total bacterial and mold counts.
2. *Nutrient Gelatin* for obtaining the liquefier counts.
3. *Modified Ashby's Agar*[1] for obtaining the *Azotobacter*, *Ps. radicicola* and *Nocardia* counts.

SOIL DILUTIONS

After thoroughly mixing the soil samples and discarding any gravel or lumps, 20 gm. of the remaining soil were weighed out on a piece of flamed copperfoil, and put into a 500-cc. Erlenmeyer flask containing 200 cc. of sterile water.

This was shaken for 5 minutes and after the coarser particles had settled, 10 cc. of the mixture was transferred to a 90-cc. sterile water blank which was thoroughly shaken, and from this 10 cc. was transferred to another 90-cc. water blank and further dilutions were made in the same way until a series of 1/10, 1/100, 1/1,000, 1/10,000, 1/100,000 and 1/1,000,000 gram of soil

[1] Ashby's agar was modified by using 10 gm. of cane sugar in place of 20 gm. of mannite.

per cubic centimeter were obtained. In general the higher dilutions were used for the total counts of the surface soils, and the lower dilutions for the subsoils.

The prevailing types of colonies were isolated from the various plate cultures on the tubes of similar medium to that of the plate from which they were taken. These tube cultures were then used for making microscopic preparations for describing the organisms.

The counts recorded are the average counts from plate cultures made from three dilutions of each soil sample.

The *Ps. radicicola* counts are only approximate, as the colony appearance of some other species of bacteria rather closely resembles that of *Ps. radicicola.*

TABLE 1

Soil type 1—Yellow sand

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	1	Typical	373,000	413,000	0	0	20,000	120,000
Sub.	2		433,000	403,000	0	0	30,000	65,000
Sur.	4	Finer sand, simi-	380,000	10,000	0	0	100,000	140,000
Sub.	5	lar to type 2	340,000	2,000	0	0	400,000	65,000

TABLE 2

Soil type 2—Light to medium brown, fine sand and sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	7	Representing the	910,000	56,000	0	0	300,000	190,000
Sub.	8	fine sand. Clay subsoil	950,000	1,000	0	0	100,000	100,000
Sur.	10	Same field as 7–8	273,000	24,000	0	0	200,000	5,500
Sub.	11	but taken from hollows	200,000	2,000	0	0	0	10,000
Sur.	13	Shallow, not so	740,000	77,000	0	100,000	300,000	500,000
Sub.	14	sandy. Clay subsoil of 13	1,300,000	5,000	0	100,000	200,000	130,000
Sur.	17	Representing the	810,000	46,000	0	200,000	600,000	80,000
Sub.	18	fine sandy loam	270,000	8,000	0	0	0	60,000
Sur.	20	Typical. Same as	140,000	19,000	0	70,000	240,000	10,000
Sub.	21	17–18	433,000	8,000	0	30,000	80,000	25,000
Sur.	23	Same as 13–14	2,150,000	100.000	0	0	200,000	670,00
Sub.	24	but clay subsoil of 8	1,880,000	15,000	0	400,000	0	160,00

TABLE 3

Soil type 3—Light to medium brown, gravelly, sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	26	Typical	2,650,000	105,000	195	900,000	600,000	20,000
Sub.	27		3,350,000	27,000	75	1,200,000	800,000	185,000
Sur.	29	Flat, sand	2,270,000	240,000	40	500,000	200,000	130,000
Sub.	30		1,110,000	38,000	20	700,000	200,000	60,000
Sur.	32	Typical	1,750,000	91,000	0	200,000	100,000	1,200,000
Sub.	33		1,000,000	54,000	0	200,000	100,000	470,000
Sur.	35	Typical	1,440,000	41,000	10	200,000	400,000	260,000
Sub.	36		650,000	13,000	0	0	200,000	320,000

TABLE 4

Soil type 4—Medium brown loam, in which stones vary in size and quantity. Variable gravel content

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	38	Typical of medium soil	1,730,000	12,000	1,710	600,000	1,400,000	40,000
Sub.	39		1,110,000	55,000	40	100,000	200,000	100,000
Sur.	41	Less stone and gravel in surface, more clay in subsoil	3,780,000	30,500	230	1,000,000	700,000	0
Sub.	42		2,980,000	210,000	0	200,000	600,000	10,000
Sur.	44	Probably belongs to type 3	950,000	355,000	0	100,000	200,000	0
Sub.	45		1,020,000	25,500	0	600,000	300,000	0
Sur.	47	Shallow soil, hard-pan or boulder, clay subsoil	1,030,000	64,500	170	400,000	800,000	30,000
Sub.	48		513,000	64,500	120	0	100,000	80,000
Sur.	49	About the same as 38–39	3,270,000	66,000	520	400,000	300,000	200,000
Sub.	50		1,700,000	79,000	80	1,200,000	200,000	240,000
Sur.	52	Slightly more gravel than 49–50 and 38–39	3,300,000	155,000	345	400,000	900,000	550,000
Sub.	53		2,330,000	95,000	70	300,000	200,000	295,000

TABLE 5

Soil type 5—Black sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	55	Typical	3,970,000	260,000	410	200,000	1,300,000	140,000
Sub.	56		255,000	125,000	25	800,000	200,000	5,000
Sur.	58	Lighter in color than typical soil	4,770,000	93,000	210	400,000	200,000	340,000
Sub.	59		610,000	55,000	0	200,000	0	55,000
Sur.	61	Typical	1,610,000	63,000	10	0	400,000	65,000
Sub.	62		1,320,000	8,500	10	200,000	0	22,000
Sur.	64	Same area as 58–59, practically belongs to type 2	2,230,000	53,000	0	0	100,000	21,500
Sub.	65		540,000	8,500	0	0	0	65,000

TABLE 6

Soil type 6—Sandy clay loam, usually dark brown

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	67	Typical except that the sand is of finer grade than usual	2,870,000	61,000	660	1,900,000	300,000	10,000
Sub.	68		1,830,000	55,000	80	1,600,000	0	0
Sur.	70	Probably belongs to type 8	4,100,000	160,000	85	800,000	400,000	40,000
Sub.	71		650,000	15,500	0	400,000	0	20,000
Sur.	73	Typical	3,750,000	190,000	1,800	T.M.	T.M.	0
Sub.	74		3,250,000	185,000	125	T.M.	T.M.	0

TABLE 7

Soil type 7—Brown, and usually moderately stony, clay loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	76	Lighter than typical soil	3,700,000	170,000	970	700,000	1,000,000	170,000
Sub.	77		2,500,000	226,000	850	800,000	600,000	75,000
Sur.	79	Heavier than typical soil	9,100,000	68,000	60	5,000,000	700,000	230,000
Sub.	80		4,600,000	67,000	0	3,500,000	200,000	260,000
Sur.	82	Typical	4,400,000	750,000	120	1,000,000	1,500,000	20,000
Sub.	83		3,400,000	900,000	75	600,000	850,000	15,000
Sur.	85	Typical	8,500,000	122,000	0	500,000	1,900,000	245,000
Sub.	86		1,670,000	4,000	0	1,000,000	100,000	210,000

TABLE 8

Soil type 8—Very fine, sandy, heavy clay loam and clay, usually dark gray to black

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	88	Typical	6,980,000	385,000	90	1,500,000	1,200,000	195,000
Sub.	89		666,000	20,500	0	400,000	100,000	65,000
Sur.	91	Surface a little lighter than 88–89	2,680,000	40,000	0	200,000	100,000	20,000
Sub.	92		1,020,000	20,500	0	300,000	0	0
Sur.	94	Typical, but not so old; higher nitrogen content	5,100,000	76,000	0	1,400,000	700,000	135,000
Sub.	95		690,000	4,000	0	0	0	30,000

TABLE 9

Soil type 9—Muck and peat with clay subsoil

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	97	Typical	5,270,000	850,000	0	700,000	1,900,000	75,000
Sub.	98		880,000	28,500	0	90,000	70,000	200,000

TABLE 10

Soil type 10—Very fine, sandy, silty loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	100		1,700,000	47,000	230	500,000	0	185,000
Sub.	101		155,000	12,000	0	300,000	100,000	150,000
Sur.	103	Typical	2,300,000	650,000	770	480,000	620,000	25,000
Sub.	104		1,060,000	390,000	200	960,000	350,000	30,000

TABLE 11

Soil type 11—Silty loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	106	Typical	385,000	280,000	60	120,000	350,000	260,000
Sub.	107		640,000	60,500	0	220,000	200,000	75,000

TABLE 12

Soil type 12—Red shale loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	112	Typical	3,150,000	585,000	0	270,000	830,000	75,000
Sub.	113		3,900,000	250,000	0	450,000	T.M.	95,000

TABLE 13

Soil type 13—Brownish red clay (shale)

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	114	An average sample of the large areas belonging to this type, though color not quite typical	1,780,000	26,500	0	300,000	700,000	30,000
Sub.	115		1,720,000	9,000	0	0	700,000	80,000
Sur.	117	Typical	9,800,000	155,000	0	T.M.	T.M.	250,000
Sub.	118		14,400,000	59,000	0	T.M.	T.M.	1,000

TABLE 14

Soil type 14—Like type 5 in texture, but belongs to a shale series

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	120	Typical	4,200,000	105,000	0	700,000	T.M.	0
Sub.	121		5,300,000	280,000	0	800,000	T.M.	0

TABLE 15

Soil type 15—Dark gray to black, shale clay

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	123	Typical	2,100,000	180,000	0	380,000	640,000	150,000
Sub.	124		4,100,000	38,000	0	160,000	730,000	10,000
Sur.	126	Also typical, but from an area that has not been cultivated so long as 123–124; more organic matter	4,180,000	93,000	0	0	700,000	230,000
Sub.	127		1,730,000	10,500	0	0	200,000	190,000

TABLE 16

Soil type 16—Gray shale loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	129	A brown shale loam has more organic matter and is a good soil	4,830,000	435,000	0	1,000,000	800,000	120,000
Sub.	130		750,000	83,000	0	0	0	180,000
Sur.	132	Typical	3,000,000	57,000	0	580,000	400,000	200,000
Sub.	133		1,870,000	12,000	0	620,000	240,000	240,000

TABLE 17

Soil type 17—Stony, fine sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	135	Typical of best phase; inconveniently stony but passable as a soil	7,400,000	425,000	970	150,000	350,000	270,000
Sub.	136		4,200,000	50,000	295	420,000	190,000	320,000
Sur.	138	Bouldery and stony, only fit for pasture for sheep	3,500,000	47,000	0	900,000	600,000	315,000
Sub.	139		2,100,000	100,000	0	300,000	300,000	120,000

DISCUSSION

Relation of Azotobacter, the principal nitrogen-fixing species of soil bacteria, to the soil types

It will be seen from the above counts that *Azotobacter* were found present in 9 out of the 17 soil types examined and in 22 out of the 29 soil samples that represented these 9 types. These types included all the loams except the shale loam as follows:

SOIL TYPE NUMBER	SOIL TYPE	PRESENCE OF AZOTOBACTER
3	Light brown gravelly sandy loam	3 out of 4 samples gave Azotobacter
4	Medium brown loam, stone varying in size and quantity	5 out of 6 samples gave Azotobacter
5	Black sandy loam, clay subsoil	3 out of 4 samples gave Azotobacter
6	Sandy clay loam	3 out of 3 samples gave Azotobacter
7	Brown, moderately strong clay loam	3 out of 4 samples gave Azotobacter
8	Fine sandy heavy clay loam	1 out of 3 samples gave Azotobacter
10	Fine sandy silty loam	1 out of 3 samples gave Azotobacter
11	Silty loam	1 out of 1 sample gave Azotobacter
17	Stony fine sandy loam	1 out of 2 samples gave Azotobacter

On the other hand, no *Azotobacter* were found in the remaining 8 soil types which included the sand, peat-muck and shale soils as follows:

SOIL TYPE NUMBER	SOIL TYPE	ABSENCE OF AZOTOBACTER
1	Yellow sand	2 samples
2	Light brown sand and sandy loam	6 samples
9	Muck and peat with clay subsoil	1 sample
12	Red shale loam	1 sample
13	Brownish red clay shale	2 samples
14	Brownish red clay shale, but sandy	1 sample
15	Dark gray to black shale clay	2 samples
16	Gray shale loam	2 samples

We assume from the above that in all probability the light sandy soils had not sufficient organic matter present and the peat-muck and shale soils were too strongly acid to favor the development of the *Azotobacter* nitrogen-fixing bacteria.

Relation of Ps. radicicola, the legume root nodule bacteria, to the soil types

As the colony appearance of *Ps. radicicola* is rather closely simulated by several other species of bacteria, we cannot be sure that all the counts above recorded as *radicicola* are true members of this species, although every care was taken to discard any that were not true to type.

It is interesting to note that every soil type examined, with the exception of one only, i.e., type 1, yellow sand, had a fairly high *Ps. radicicola* content, and further, that in most cases the sub-surface samples had a higher *radicicola* content than the surface samples. This is as one might expect, as the *Ps. radicicola* would come largely from the decayed legume root nodules.

In general, the soils that were favorable for *Azotobacter* had the higher *radicicola* counts, but the fact that fairly high *radicicola* counts were obtained in the peat-muck and shale samples, which were presumably too acid for *Azotobacter* would indicate that some varieties of *Ps. radicicola* at least had a fair degree of toleration for acid soils.

We could not determine from a bacterial culture examination what different varieties of *Ps. radicicola* were present, as the different varieties are determined by the different varieties of legume with which they will associate.

Therefore, we cannot say whether or not all varieties of legumes could be grown satisfactorily on all these different soils, even though *Ps. radicicola* was found present in considerable numbers, as these numbers may represent only a limited number of legume varieties, thus limiting the choice of legumes to those varieties with which the varieties of *Ps. radicicola* found would associate.

Relation of the total bacterial count and liquefier count to the soil types

In general the total bacterial count and liquefier count represent the majority of bacterial species that are active in digesting and preparing the crude plant-food present in the soil, thereby rendering it available in a suitable condition for assimilation by the growing crops. The number found is considered in a general way as being indicative of soil fertility, as their action is essential in the preparation of the plant-food in the soil.

With the exception of sample 1, yellow sand, sample 2, light brown sand, and sample 11, silty loam, each of which has a very low total count, all the samples have a fair bacterial content for arable soils. It is interesting to note that the shales have as high a total count as the loams; as a matter of fact one shale, no. 13, has the highest count of all. This would indicate that while the shale and peat muck soils examined were not suitable for the nitrogen-fixing *Azotobacter*, they were about equally suitable with the loams for the species of the plant-food digesting bacteria.

Relation of Nocardia (Actinomycetes) count to the soil types

It is considered (J. Conn) that the *Nocardia* (*Actinomycetes*) or thread bacteria, found in soil are largely active in bringing about the decay of dead root fibers, particularly those of sod, thereby aiding in preparing them for food of growing crops.

The counts above recorded show that they were least numerous in the sand but averaged up much alike in the loams, peat muck and shales, in all of which they were fairly numerous.

Molds

It is not at present considered that molds have any important rôle to play in connection with soil fertility. It is, however, interesting to note from the above counts that type 1, the yellow sand which gave no *Azotobacter*, no *radicicola* and the lowest of all total bacterial counts, gave a fairly high mold count, while type 6, sandy clay loam, which gave the highest *Azotobacter* count, a very high *radicicola* and total bacteria count, showed no molds. With one other exception, type 14, a sandy clay shale, which also gave no molds, the mold content of the samples was fairly uniform.

A chemical and physical analysis of these soils is being made, respectively, in the chemistry and physics departments of the college.

STATEMENT OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC. REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

Of SOIL SCIENCE, published monthly at Baltimore, Maryland, for October 1, 1919.

STATE OF NEW JERSEY } ss.
COUNTY OF MIDDLESEX }

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Carl R. Woodward, who, having been duly sworn according to law, deposes and says that he is the Assistant Editor of SOIL SCIENCE and that the following is, to the best of his knowledge and belief a true statement of the ownership, management (and if a daily paper, the circulation), etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, printed on the reverse of this form, to wit:

1. That the names and addresses of the publisher, editor, managing editor, and business managers are:

Publisher, Williams and Wilkins Company, Mt. Royal and Guilford Aves., Baltimore, Maryland.

Editor, Jacob G. Lipman, College Farm, New Brunswick, New Jersey.

Managing Editor, None.

Business Managers, None.

2. That the owners are: Trustees of Rutgers College, New Brunswick, New Jersey.

3. That the known bondholders, mortgagees, and other security holders owning or holding 1 per cent or more of total amount of bonds, mortgages, or other securities are: None.

CARL R. WOODWARD, *Assistant Editor.*

Sworn to and subscribed before me this 19th day of September, 1919.

RUSSELL E. LONG, *Notary Public.*

[SEAL] My commission expires January 31, 1924.